*Pol. J. Environ. Stud. Vol. 21, No. 1 (2012), 15-21*

*Review*

# *N*-Acylhomoserine Lactones (AHLs) as Phenotype Control Factors Produced by Gram-Negative Bacteria in Natural Ecosystems

**Kamila Myszka*, Katarzyna Czaczyk**

Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Wojska Polskiego 48, Poznań, Poland

*Received: 2 April 2011*
*Accepted: 21 July 2011*

**Abstract**

Bacteria are able to sense an increase in cell population density and to respond to it by the induction of a particular set of genes. This mechanism, called *quorum sensing*, includes in gram-negative bacteria the production and secretion of an acyl homoserine lactone, which diffuses through the cell wall, from the cell to the medium. Bacteria use the *quorum sensing* mechanism to regulate a variety of phenotype, such as bioluminescence, virulence factor production, biofilm formation, and motility, which are essential for the successful establishment of a symbiotic or pathogenic relationship with a eukaryotic host. Understanding of mechanism of *quorum sensing* creates the possibility of elaborating new drugs efficient against pathogens. It also suggests ideal targets for agricultural application of bacteria. In the review the basic concepts concerning the bacterial *quorum sensing* mechanism were discussed. In this paper some aspects of eukaryote-bacterial cross talk in natural environment were also described.

**Keywords:** *quorum sensing*, acyl homoserine lactones, AHL, signal molecules

## Introduction

Bacteria have evolved to exist in complex communities. Within these consortia, bacteria are able to communicate with each other to regulate gene expression according to population density in a process that has been termed *quorum sensing*, a mechanism that has evolved in particular microorganisms to function in a particular ecosystem and circumstances [1].

*Quorum* size is sensed by the bacteria through the production of small diffusible molecules that accumulate in their surroundings as the population increases. When the concentration of the molecule exceeds the threshold value, signaling pathways are activated (or de-repressed) and the bacteria respond by altering gene expression [2]. In gram-negative bacteria, a large number of *quorum sensing* systems have acyl homoserine lactone molecules (AHL) as signals that activate a transcriptional regulatory protein [3, 4]. By contrast, *quorum sensing* mechanisms in gram-positive bacteria typically use secreted peptides as signal molecules and a two-component regulatory system (composed of a membrane-bound histidine kinase receptor and an intracellular response regulator) to detect the peptide and trigger the required changes in gene expression [5, 6].

Many saprotrophic and pathogenic gram-negative and gram-positive bacteria, including the genera: *Agrobacterium*, *Aeromonas*, *Bacillus*, *Brukholderia*, *Chromobacterium*, *Citrobacter*, *Enterobacter*, *Enterococcus*, *Erwinia*, *Hafnia*, *Klebsiella*, *Nitrosomonas*, *Obesumbacterium*, *Pantoea*, *Pseudomonas*, *Rahnella*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Serratia*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, *Vibrio*, *Xenorhabdus*, and *Yersinia* utilize the

*e-mail: kmyszka@up.poznan.pl

Table 1. Some examples of signal molecules produced by gram-negative bacteria.

| Microorganism | Major AHL(s) | References |
|---|---|---|
| *Aeromonas hydrophila* | C4-HSL | [59] |
| *Erwinia carotovora* ssp. *carotovora* | 3-oxo-C6-HSL | [2] |
| *Pantoea (Erwinia) stewartii* | 3-oxo-C6-HSL | [2] |
| *Pseudomonas aeruginosa* | C4-HSL 3-oxo-C12-HSL | [15] |
| *Pseudomonas putida* | 3-oxo-C10-HSL 3-oxo-C12-HSL | [60] |
| *Serratia liquefaciens* | C4-HSL | [58] |
| Serratia marcescens | C6-HSL 3-oxo-C6-HSL | [58] |
| *Vibrio fischeri* | 3-oxo-C6-HSL | [39] |

*quorum sensing* system to regulate metabolic activity [2, 5, 7]. This phenomenon was also observed in extermophilic microorganisms, such as the haloalkaliphilic archeon *Natronococcus occultus* or the acidophilic proteobacterium *Acidithiobacillus ferrooxidans* [8, 9].

Literature mostly evidences mechanisms by which gram-negative bacteria monocultures communicate in artificial ecosystems created in a laboratory. However, in the natural environment most bacterial species do not live in isolation and consequently it is the impact of *quorum sensing* molecules on both other microorganisms and higher organisms. In this review, we briefly overview the acyl homoserine lactone-based *quorum sensing* mechanism of gram-negative bacteria and focus on eukaryote-bacterial cross talk in natural environment.

## Basics of *Quorum Sensing* in Gram-Negative Bacteria

The *quorum sensing* system in gram-negative bacteria consists of:

- signal molecules (autoinducers)
- autoinducer synthases LuxI
- LuxR-type regulators
- target genes [3, 4].

Intracellular transfer of information between bacteria belonging to the same or different species, genera and even families is carried out with the help of acyl homoserine lactones [4, 10]. AHL molecules have a conserved homoserine lactone (HSL) ring that is acylated with a fatty acyl group at the $\alpha$-position. AHL may contain C4 to C18 acyl side chains and, at the third carbon, either an oxo, or a hydroxyl, or no substitution. Based on the length of the acyl groups, AHL are classified as short- or long-chain molecules [11].

The best investigated signal molecules produced by gram-negative bacteria are AHL with C6 and C8 acyl chains [11]. C4-HSL and 3-hydroxy-C4-HSL are the shortest AHLs found naturally [12]. AHL with C14 and C18 acyl chains contain one or two double bonds [13, 14]. Examples of different signal molecules produced by gram-negative bacteria are listed in Table 1.

According to Yates et al. [15] the pH value directly influenced the structure and properties of bacterial signal molecules. The HSL ring is highly susceptible to pH-dependent ring opening. For C3-HSL molecules this was observed below the pH value of 7. The conformal changes of AHLs are noticed when the pH value of the growth environment is decreased to 6 and 5. Ring-opened AHLs were not active as *quorum sensing* signals. Yates at al. [15] also demonstrated that the susceptibility of the HSL ring to pH value is decreased as the acyl side chain is lengthened [15].

The bacteria membrane is permeable to AHLs, and thus, these signal molecules freely diffuse into and out of the cell to the surrounding environment. The transport of long-chain AHLs has been also associated with efflux pump [16]. The migration process of AHLs is coupled to the length of the acyl chains. Short-chain AHLs that have 4 to 6 carbon atoms in the acyl moiety are diffused passively through cell membrane [17]. Autoinducers with C7 and -C18 acyl chains are in part actively transported *via* efflux pumps [16]. Moreover, the process of AHL migration also depends on bacterial population density in the environment [10].

LuxI is the enzyme responsible for synthesis of autoinducers in the *quorum sensing* system [10, 18, 19]. The bacterial genome includes more than 100 different LuxI homologues. In gram-negative cells, each LuxI synthase specifically catalyzes the biosynthesis of one type of AHL [10]. Moré et al. [18] demonstrated that in *Agrobacterium tumefaciens* culture purified hexahistidinyl-TraI (the LuxI homologue) can catalyse the *in vitro* synthesis of AHL. Similarly, Schaefer et al. [19] showed that in *Vibrio fischeri* population, purified a maltose binding protein-LuxI fusion polypeptide is capable of catalyzing the AHLs.

When the AHL concentration has been attained within the culture, signal molecules bind to and activate members of the LuxR transcriptional regulator protein family [2, 10]. The LuxR polypeptide contains two domains: cognate AHL interacts with LuxR through signal-binding regions in the N-terminal domain. The interaction between LuxR and autoinducer is specific in that AHL analogs show limited or no LuxR-mediated activation of the target genes [20].

Based on phylogenetic comparisions, LuxI and LuxR family members may have been acquired by lateral gene transfer [3]. A number of LuxR and LuxI homologues are located on plasmids. This was observed in the culture of *Agrobacterium* spp. (Ti plasmid) and *Rhizobium* spp. [21, 22]. Wei et al. [23] noticed that in *Serratia marcescens* a LuxRI system (termed SpnRI), which is located on a Tn3 type transposon, could be transferred between plasmids and chromosomes in *E. coli* and *S. marcescens*. Acquisition of the SpnR system by an AHL-negative *E. coli* strain lead to AHL-dependent regulation of swarming motility and pigment production by the cells [23]. This work supports the notion that lateral gene transfer might play a crucial function in the mobility of LuxRI systems between different bacterial genera.

## AHL Molecule Synthesis

Biosynthesis of AHL molecules was first described by Eberhard et al. [24] for the luminous marine bacterium *Photobacterium fischeri* (*Vibrio fischeri*). The bacteria were cultured on the medium supplemented with S-adenosylmethionine (SAM) and a fatty acids derivative. Eberhard et al. [24] observed that SAM was the amino acid substrate necessary for the synthesis of 3-oxo-C6-HSL by examined cells. Similarly, the production of 3-oxo-C6-HSL by *E. coli* depends on the presence of methionine or SAM in the medium [25]. Hanzelka and Greenberg [25] noticed that the lack of amino acids in the growth environment inhibited the methionine adenosyltransferase reaction (which converts methionine to SAM). According to Val and Cronan [26], decreasing the intracellular levels of SAM in *E. coli* reduced the efficiency of AHL signal biosynthesis by examined cells. Intracellular SAM influences on the expression of TraI (LuxI homologue) in *E. coli*.

Eberhard et al. [24] demonstrated that 3-oxohexanoyl coenzyme A or the acyl carried protein (ACP) are the donor of fatty acid side chain in the biosynthesis of 3-oxo-C5-HSL in *V. fischeri*. The work of Val and Cronan [26] demonstrated that a block of β-oxidative fatty acid in cytosol totally inhibited AHL production by examined cells. This result correlated with the study of Hoang and Schweizer [27], who investigated the idea that the grade of AHLs produced by a strain of *P. aeruginosa* carrying a mutation in the *fabI* gene (that encodes a protein arranged in fatty acid production) was significantly reduced.

In the biosynthesis of AHL signals, SAM binds to the active site of the enzyme and the appropriate acyl group is transferred to this complex from a charged ACP. Then acyl group forms an amide bond with the amino group of SAM [2]. In the *quorum sensing* mechanisms of bacteria belonging to different species, the action of complex SAM/acyl-ACP is conserved [28].

The production of autoinducers by the bacteria can also be accomplished by members of the LuxM family. These syntheases have been discovered in *Vibrio* spp. [29, 30]. *LuxM* genes are associated with genes coding for histidine protein kinase sensor. These proteins in the periplasm during the action with AHLs, trigger a phosphorelay cascade resulting to transcriptional activation of the target *quorum sensing* dependent genes [31, 32].

## Regulation of Bacterial Phenotype by *Quorum Sensing*

In the natural environment bacteria use *quorum sensing* mechanisms to regulate a variety of phenotypes such as bioluminescence, toxin secretion, degradative enzyme production, biofilm formation, and motility [2, 3]. These phenotypes are essential for the successful establishment of a symbiotic, beneficial or pathogenic relationship with a eukaryotic host [3]. In natural ecosystems coordinated expression of these phenotypes is also crucial in cell migration to a more suitable environment/better nutrient supply and in adaptation to new modes of growth, which may afford protection from deleterious environments [2, 10]. A variety of different bacterial strains can make the same AHL, but this molecule may be involved in the regulation of different phenotypes in each strain [3]. Some of these phenotypes are described below.

### Bioluminescence

Luminous bacteria are common in marine environments, and occur as free-living forms in seawater, on organic debris, as commensals in the gut of many marine animals, and as light organ symbionts. Marine bioluminescent bacteria are members of the genera *Photobacterium*, *Vibrio*, and *Xenorhabdus* [33]. The regulation of bacterial bioluminescence is mediated by a *quorum sensing* system [34]. In *V. fischeri* the bioluminescence gene cluster consist of eight genes (*luxA-E*, *luxG*, *luxI*, *luxR*) arranged in two bi-directionally transcribed operons [35]. The products of the *luxI* and *luxR* genes act as regulators of the process of bioluminescence in *Vibrio* spp. culture [36]. The subunits of the heterodimeric luciferase enzyme (α and β) are the products of the *luxA* and *luxB* genes. Luciferase catalyses the oxidation of reduced flavin mononucleotide and a long-chain aliphatic aldehyde. Blue-green light with a wavelength of about 490 nm is simultaneously emitted because the generation of an intermediate molecule is an electronically excited state [29, 33]. *luxC* and *luxE* encode products forming a complex of enzyme, responsible for the synthesis of the aldehyde substrate utilized by the luciferase [37]. According to Zenno and Saigo [38], *luxG* probably encodes a flavin reductase.

In *Vibrio* spp. the bioluminescence is activated by 3-oxo-C6-HSL molecules. Kaplan and Greenberg [17] observed that the concentration of 3-oxo-C5-HSL at the level of 10 nM induces the expression of *luxI* gene in *Vibrio* spp. cells. 3-oxo-C6-HSL molecule interacts with the transcriptional regulator protein, LuxR. The activated protein, LuxR, binds to a 20-bp DNA element, known as the *lux* box [39]. From there, LuxR induces transcription of *luxICDABEG*. Consequently, the cellular levels of mRNA transcripts encoding both bioluminescence and the production of 3-oxo-C6-HSL increase considerably. The newly

synthesized 3-oxo-C6-HSL molecules then also bind to N-terminal region of LuxR in neighboring *Vibrio fischeri* cells. This autoinduction mechanism have an effect on the bioluminescence until the signal molecules have achieved a concentration of 100nM [40].

At low levels of 3-oxo-C6-HSL in the surrounding environment, LuxR protein induces the transcription of *luxR*. When 3-oxo-C6-HSL is abundant, activated LuxR blocks out the transcription of *luxR* [41]. This mechanism is unknown. However, the repression of *luxR* transcription is dependent on the presence of the *lux* box element situated within *luxD*. According to Shadel and Baldwin [41], the *lux* box element functions as autoregulator of bioluminescence in *Vibrio* spp.

## Extracellular Virulence Factors

In bacteria that are widespread in natural environments, the *quorum sensing* mechanism controls cell determinants of pathogenicity. This was observed in the members of *Pseudomonas* spp., *Erwinia* spp., and *Agrobacterium* spp. that cause injury and tumor formation on poplar stems [42].

*Quorum sensing* of *P. aeruginosa* encompasses two pairs of LuxRI homologues: LasRI and RhlRI [43, 44]. The major signal molecules synthesized *via* LasI and RhlI, respectively, are 3-oxo-C12-HSL and C4-HSL. *P. aeruginosa* employs a multilayered hierarchical *quorum sensing* cascade linking LasR-3-oxo-C12-HSL and RhlR-C4-HSL to integrate the regulation of virulence determinants with survival with stationary phase [10]. According to You et al. [45], the expression of LasI is very sensitive to the LasR-3-oxo-C12-HSL level. An increase in LasI production leads to a rapid increase in 3-oxo-C12-HSL [46]. Furthermore, LasR-3-oxo-C12-HSL is required for the expression of the *rhlRI* locus [10]. *rhlRI* encoding a rhamnosyltransferase is required for the production of rhamnolipid [47]. The presence of these biosurfactants reduces surface tension and allows bacteria to swarm over semi-solid surfaces [48].

Many species of Erwinia have been found to produce AHLs. C6-HSL, 3-oxo-C6-HSL, and C10-HSL are reported from *Erwinia chrysanthemi* and *Pantoea stewartii* subsp. *stewartii* [49]. The *quorum sensing* system of *P. stewartii* subsp. *stewartii* consists of EsaI (LuxI homologue), which directs production of 3-oxo-C6-HSL and a EsaR (LuxR homologue) [50, 51]. 3-oxo-C6-HSL is required for the cell density-dependent production of an extracellular virulence determinant, called stewartan. This compound can block plant xylem vessels and cause wilting [51].

A *Quorum sensing* mechanism is also used to regulate the expression of pathogenicity determinants of *A. tumefaciens*. The main role of AHL signaling found to date is in regulating the initiation of conjugation and the transfer of tumor-inducing (Ti) virulence plasmid to a Ti-plasmidless saprophytic *A. tumefaciens* recipient in the tumoursphere [52]. Conjugation is induced when a TraR (a homologue of LuxR) binds its cognate AHL (3-oxo-C8-HSL), synthesized by the *traI* gene product (a homologue of LuxI) [53]. In the absence of AHLs, TraR is present as insoluble monomers that are rapidly degraded by proteases. Whereas the presence of signal molecules induces the process of soluble TraR formation. TraR proteins are resistant to proteases and are capable of transcription activation after binding to conserved 18 bp target sequences [52]. Qin et al. [54] observed that before AHL binding, the fat-soluble TraR are found in cytoplasmic membranes of *A. tumefaciens*, whereas after exposure to 3-oxo-C8-HS, AHL-containing TraR are found in the cytoplasm. It was suggested that a membrane location for the TraR monomer may favour interaction with externally sourced signal molecules [54].

## Biofilm Development

In the natural environment a *quorum sensing* mechanism regulates the bacterial colonization process of solid materials. The production and secretion of AHL signals induces a reversible attachment of planktonic microorganisms to solid surfaces [55]. The AHLs diffuse radially away from the floating cell. When the cell starts anchoring to the surface, diffusion of AHLs is limited from one side. This process allows the cell to sense that it is near the surface [56].

In a mature biofilm, the diffusion process of AHLs between the bacteria is unlimited [10]. In contrast to planktonic populations, in biofilms the contact between the cells is more probable. Moreover, in biofilms there are shorter-distance migration of AHL molecules [2]. The role of AHL-mediated *quorum sensing* in biofilm formation has been demonstrated for *Burkholderia cenocepacia*, *Aeromonas hydrophila*, *P. aeruginosa*, *P. putida*, and *S. marcescens* [57-60]. AHL-negative mutants of *P. aeruginosa*, *B. cenocepacia,* and *A. hydrophila* showed defects in the late stage of biofilm development and thus were unable to form biofilms with a wild-type architecture. The structure of natural biofilms is heterogenous and consists of microcolonies divided by the water channel systems [57-61].

The main structural components of microcolonies of biofilm are extracellular polysaccharides [62]. The production of extracellular alginate in *P. aeruginosa* PA14 biofilms is induced by 3-oxo-C12-HSL [13]. *S. marcescens* MG1 synthesizes an exopolysaccharides in a C4-HSL-dependent manner. This type of polymer contains β 1-3- or β 1-4 glycosidic linkages. This exopolysaccharide promotes attachment of *S. marcescens* to abiotic materials in the first stages of biofilm development. This is in contrast to what is found in most other bacteria populations, where *quorum sensing* stimulates extracellular polysaccharides production at later stages of biofilm maturation [58].

The study of Torres et al. [63] suggests that bacteria may use *quorum sensing* to trigger biofilm dispersal when the biofilm population reaches a critical size. Such *quorum sensing*-dependent matrix degradation is well documented for *P. aeruginosa* The released microorganisms may be transported to newer locations and again restart the biofilm formation process [64].

## *Quorum Sensing* in Natural Environment

In natural environments many gram-negative bacteria found in association with higher organisms have been demonstrated to utilize a *quorum sensing* mechanism. The list includes epiphytic, pathogenic, and rhizosphere-inhabiting bacteria, as well as nitrogen-fixing symbionts [65]. However, our current understanding of the *in vivo* role is still fragmentary, with most of the information based on *in vitro* experiments. Researchers have been focused so far on *quorum sensing* interference actions.

In natural environments, higher organisms and bacteria coexisting in the same niches evolved to sense each other's presence. These interspecies and interkingdom communications contain several activities that stimulate or inhibit AHL-regulated bacteria behaviours. The first demonstrated example of higher organism secreting metabolites that specifically interfere with bacterial signal molecules was a macroalga called *Delisea pulchra*. *D. pulchra* synthesized furanones, which compete with cognate AHL molecules for the binding site on receptor protein and therefore prevent activation of the target gene expression [66]. A study by Manefield et al. [67] showed that furanones are capable of displacing radiolabelled C5-HSL from the surface of *E. coli* that are overexpressing LuxR. Furanones produced by *D. pulchra* were able to inhibit AHL-dependent extracellular plant cell walls degrading enzyme production in *Pectobacterium carotovorum* subsp. *carotovorum* [67]. Further analysis of these molecules will indicate whether they are related to the furanones or whether they belonging to a distinct group of AHL mimics [68]. Structural characterization of AHL mimic compounds can have important effects on bacterial colonization and infection of host organisms in the natural environment.

*Quorum sensing* interference actions was also observed between the green seaweed *Enteromorpha* and bacteria of the species of *V. anguillarum* [69]. Zoospores of the eukaryote seaweed can exploit the bacterial sensory mechanism. Joint et al. [69] showed that Enteromorpha is attracted by AHL molecules to settle on the bacterial biofilms consisting of *V. anguillarum* cells. However, the way in which zoospores identify and respond to bacterial signal molecules is still unknown. Interruption of the *quorum sensing* mechanism of *Vibrio* spp. may provide the tools to control this fouling organism in natural ecosystems.

Higher plants have also evolved strategies to interfere with the bacteria's *quorum sensing* system to prevent them from initiating a pathogenic attack. Such interference could include the production of signal blockers. Fray et al. [70] have found compounds with signal-inhibiting properties in extracts from grape and strawberry. It appears that plants use these compounds *in vivo* to inhibit bacterial cell attachment responses, thus preventing the build-up of bacterial biofilms [70]. Similar effects were observed by Teplitsky et al. [68]: authors demonstrated that higher plants such as pea and tomato produced unidentified compounds interacting with AHL-dependent *quorum sensing* system of bacteria. In this case, the strategy of blocking *quorum sensing* molecules by higher plants reduces the susceptibility of plants to bacterial infections.

In the environment of the soil, *Rhizobium* spp., *Sinorhizobium* spp., and *Bradyrhizobium* spp. use the AHL-mediated *quorum sensing* system for controlling plant nodulation. Nodulation is important in agriculture to increase crop production in areas of poor soil nitrogen availability. This process is the only possibility to bind airborne nitrogen, transform it into inorganic salts, and ensure its utilization. Marketon and Gonzalez [71] noticed that nodulation did not occur if *quorum sensing* was suppressed in these bacteria.

In conclusion, the phenomenon of a *quorum sensing* mechanism requires further *in vivo* studies. Despite intensive investigations into different aspects of *quorum sensing* systems, cell-to-cell communications have been studied in detail in only a small number of bacteria. It is often unclear why and how bacteria react to the chemical substances produced by other bacteria or the host organism, and what are the molecular basis of autoinducers (speed of evolution, quantity of substances produced, specificity of the chemical and so on). A better understanding of the factors affecting the cell-to-cell communication process (especially eukaryote-bacterial cross talk) will create capabilities for drug design as well as agricultural applications.

## References


1. DECHO A.W., NORMAN R.S., VISSCHER P.T. *Quorum sensing* in natural environments: emerging views from microbial mats. Trends Microbiol. **18**, 73, **2010**.
2. WHITEHEAD N.A., BARNARD A.M.L., SLATER H., SIMPSON N.J.L, SALMOND G.P.C. *Quorum-sensing* in Gram-negative bacteria. FEMS Microbiol. Rev. **25**, 365, **2001**.
3. GONZÁLEZ J.E., KESHAVAN N.D. Messing with bacterial *quorum sensing*. Microbiol. Mol. Biol. Rev. **70**, 859, **2006**.
4. TUROVSKIY Y., KASHTANOV D., PASKHOVER B., CHIKINDAS M.L. *Quorum sensing*: fact, fiction and everything in between. Adv. Appl. Microbiol. **62**, 191, **2007**.
5. KLEEREBEZEM M., QUADRI L.E.N., KUIPERS O.P., DE VOS W.M. *Quorum sensing* by peptide pheromones and two-component signal-transduction systems in gram-positive bacteria. Mol. Microbiol. **24**, 895, **1997**.
6. SUNTHARALINGAM P., CVITKOVITCH D.G. *Quorum sensing* in streptococcal biofilm formation. TRENDS Microbiol. **13**, 3, **2005**.
7. EBERL L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. Syst. Appl. Microbiol. **22**, 493, **1999**.
8. PAGGI R.A., MARTONE C.B., FUQUA C., DE CASTRO R.E. Detection of *quorum sensing* signals in the haloalkaliphilic archeon *Natronococcus occultus*. FEMS Microbiol. Lett. **221**, 49, **2003**.
9. RIVAS M., SEEGER M., JEDLICKI E., HOLMES D.S. Second acyl-homoserine lactone producing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Appl. Environ. Microbiol. **73**, 3225, **2007**.
10. WILLIAMS P., WINZER K., CHAN W. C., CÁMARA M. Look who's talking: communication and *quorum sensing* in the bacterial world. Phil. Trans. R. Soc. B. **362**, 1119, **2007**.

11. LITHGOW J.K., WILKINSON A., HARDMAN A., RODELAS B., WISNIEWSKI-DYE F., WILLIAMS P., DOWNIE J.A. The regulatory locus cinRI in *Rhizobium leguminosarum* controls a network of wuorum-sensing loci. Mol. Microbiol. **37**, 81, **2000**.
12. WINSON M.K., CÁMARA M., LATIFI A., FOGLINO M., CHHABRA S.R., DAYKIN M., BALLY M., CHAPON V., SALMOND G. P., BYCROFT B. W. Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA. **92**, 9427, **1995**.
13. SAKURAGI Y., KOLTER R. *Quorum-sensing* regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. J. Bacteriol. **189**, 5383, **2007**.
14. WAGNER-DÖBLER I., THIEL V., EBERL L., ALLGAIER M., BODOR A., MEYER S., EBNER S., HENNING A., PUKALL R., SCHULZ S. Discovery of complex mixtures of novel long-chain *quorum sensing* signals in free-living and host-associated marine alphaproteobacteria. Chem.-Biochem. **6**, 2195, **2005**.
15. YATES E.A., PHILIPP B., BUCKLEY C., ATKINSON S., CHHABRA S.R., SOCKETT R.E., GOLDNER M., DESSAUX Y., CÁMARA M., SMITH H., WILLIAMS P. *N*-acylhomoserine lactones undergo lactonolysis in a pH-, temperature- and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. Infect. Immun. **70**, 5635, **2002**.
16. PEARSON J.P., VAN DELDEN C., IGLEWSKI B.H. Active efflux and diffusion are involved in transport of *Psedomonas aeruginosa* cell-to-cell signals. J. Bacteriol. **181**, 1203, **1999**.
17. KAPLAN H.B., GREENBERG E.P. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. J. Bacteriol. **163**, 1210, **1985**.
18. MORÉ M.I., FINGER L.D., STRYKER J.L., FUQUA C., EBERHARD A., WINANS S.C. Enzymatic synthesis of a *quorum-sensing* autoinducer through use of defined substrates. Sciences. **272**, 1655, **1996**.
19. SCHAEFER A.L., VAL D.L., HANZELKA B.L., CRONAN J.E., GREENBERG E.P. Generation of cell-to-cell signals in *quorum sensing*: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. Proc. Natl. Acad. Sci. USA. **93**, 9505, **1996**.
20. FINNEY A.H., BLICK R.J., MURAKAMI K., ISHOHAMA A., STEVENS A.A.M. Role of the C-terminal domain of the alpha subunit of RNA polymerase in LuxR-dependent transcriptional activation of the *lux* operon during *quorum sensing*. J. Bacteriol. **184**, 4520, **2002**.
21. WISNIEWSKI-DYE F., DOWNIE J.A. *Quorum sensing* in *Rhizobium*. Anton van Leeuwenhoek. **81**, 397, **2003**.
22. ZHANG L., MURPHY P.J., KERR A., TATE M.E. Agrobacterium conjugation and gene regulation by N-acyl-L-homoserine lactones. Nature. **362**, 446, **1993**.
23. WEI J.R., TSAI Y.-H., HORNG Y.T., SOO P.-C., HSIEH S.C., HSUEH P.-R., HORNG J.-T., WILLIAMS P., LAI H.-C. TnTIR, a mobilevTn3-familiy transposon carring spnIR *quorum sensing* unit. J. Bacteriol. **188**, 1518, **2006**.
24. EBERHARD A., LONGIN T., WIDRIG C.A., STRANICK S.J. Synthesis of the *lux* gene autoinducer in *Vibrio fischeri* is positively autoregulated. Arch. Microbiol. **155**, 294, **1991**.
25. HANZELKA B.L., GREENBERG E.P. *Quorum sensing* in *Vibrio fischeri*: evidence that S-adenosylmethionine is the amino acid substrate for autoinducer synthesis. J. Bacteriol. **178**, 5291, **1996**.
26. VAL D.L., CRONAN J.E. *In vivo* evidence that S-adenosylmethionine and fatty acid synthesis intermediates are the substrates for the LuxI family of autoinducer synthases. J. Bacteriol. **180**, 2644, **1998**.
27. HOANG T.T., SCHWEIZER H.P. Characterization of *Pseudomonas aeruginosa* enoyl-acyl carrier protein reductase (*FabI*): a target for the antimicrobial triclosan and its role in acylated homoserine lactone synthesis. J. Bacteriol. **181**, 5489, **1999**.
28. CZAJKOWSKI R., JAFRA S. Quenching of acyl-homoserine lactone-dependent *quorum* sensing by enzymatic disruption of signal molecules. Acta Biochim. Pol. **56**, 1, **2006**.
29. HANZELKA B.L., PARSEK M.R., VAL D.L., DUNLAP P.V., CRONAN J.E., GREENBERG E.P. Acylhomoserine lactone synthase activity of the *Vibrio fischeri* AinS protein. J. Bacteriol. **181**, 5766, **1999**.
30. MILTON D.L., CHALKER V.J., KIRKE D., HARDMAN A., CÁMARA M., WILLIAMS P. The LuxM homologue VanM from *Vibrio anguillarum* directs the synthesis of N-(3-hydroxyhexanoyl) homoserine lactone nad N-hexanoyl-homoserine lactone. J. Bacteriol. **183**, 3537, **2001**.
31. CÁMARA M., WILLIAMS P., HARDMAN A. Controlling infection by tuning in and turning down the volume of bacterial small-talk. Lancet. Infect. Dis. **2**, 667, **2002**.
32. CROXATTO A., PRIDE J., HARDMAN A., WILLIAMS P., CÁMARA M., MILLTON D.L. A distinctive dual-channel *quorum-sensing* system operates in *Vibrio anguillarum*. Mol. Microbiol. **52**, 1677, **2004**.
33. BURLAGE R.S., PALUMBO A.U., HEITZER A., SAYLER G. Bioluminescent reporter bacteria detect contaminants in soil samples. Appl. Biochem. Biotechnol. **45**, 731, **1994**.
34. NEALSON K.H., PLATT T., HASTINGS J.W. Cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. **104**, 313, **1970**.
35. SWARTZMAN E., SILVERMAN M., MEIGHEN E. A. The *luxR* gene product is a transcriptional activator of the *lux* promoter. J. Bacteriol. **174**, 7490, **1992**.
36. ENGERBRECHT J., SILVERMAN M. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA. **81**, 4154, **1984**.
37. BOYLAN M., GRAHAM A.F., MEIGHEN E.A. Functional identification of the fatty acid reductase components encoded in the luminescence operon of *Vibrio fischeri*. J. Bacteriol. **163**, 1186, **1985**.
38. ZENNO S., SAIGO K. Identification of the genes encoding NAD(P)H-flavin oxidoreductases that are similar in sequence to *Escherichia coli* Fre in four species of luminous bacteria: *Photorhabdus luminescens*, *Vibrio fischeri*, *Vibrio harveyi* and *Vibrio orientalis*. J. Bacteriol. **176**, 3544, **1994**.
39. EGLAND K.A., GREENBERG E.P. *Quorum sensing* in *Vibrio fischeri*: elements of the *luxI* promoter. Mol. Microbiol. **31**, 1197, **1999**.
40. BOETTCHER K.J., RUBY E.G. Detection an quantification of *Vibrio fischeri* autoinducer from symbiotic squid light organs. J. Bacteriol. **177**, 1053, **1995**.
41. SHADEL G.S., BALDWIN T.O. Identification of a distantly locatd regulatory element in the LuxCD gene required for negative autoregulation of the *Vibrio fischeri* LuxR gene. J. Biol. Chem. **267**, 7690, **1992**.
42. MENTAG R., LUCKEVICH M., MORENCY M.-J., SÉGUIN A. Bacterial disease resistance of transgenic hybrid poplar expressing the synthetic antimicrobial peptide D4E1. Tree Physiol. **23**, 405, **2003**.

43. PASSADOR L., COOK J.M., GAMBELLO M.J., RUST L., IGLEWSKI B.H. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. Science. **260**, 1127, **1993**.
44. PEARSON J.P., PASSADOR L., IGLEWSKI B.H., GREENBERG E.P. A second *N*-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA. **92**, 1490, **1994**.
45. YOU Z., FUKUSHIMA J., ISHIWATA T., CHANG B., KURATA M., KUWAMOTO S., WILLIAMS P., OKUDA K. Purification and characterization of LasR as a DNA-binding protein. FEMS Microbiol. Lett. **142**, 301, **1996**.
46. SEED P.C., PASSADOR L., IGLEWSKI B.H. Activation of the *Pseudomonas aeruginosa* lasI gene by LasR and the *Pseudomonas autoinducer* PAI: an autoinduction regulatory hierarchy. J. Bacteriol. **177**, 654, **1995**.
47. OCHSNER U., KOCH A. K., FIECHTER A., REISER J. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. J. Bacteriol. **176**, 2044, **1994**.
48. KÖHLER T., CURTY L.K., BARJA F., VAN DELDEN C., PECHERE J.C. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J. Bacteriol. **182**, 5990, **2000**.
49. NASSER W., BOUILLANT M.L., SALMOND G., REVERCHON S. Characterization of the Erwinia chrysanthemi expI-expR locus directing the synthesis of two N-acyl-homoserine lactone signal molecules. Mol. Microbiol. **29**, 1391, **1998**.
50. BECK VON BODMAN S., FARRAND S. K. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an N-acyl homoserine lactone autoinducer. J. Bacteriol. **177**, 5000, **1995**.
51. BECK VON BODMAN S., MAJERCZAK D.R., COPLIN D.L. A negative regulator mediates *quorum-sensing* control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. Proc. Natl. Acad. Sci. USA. **95**, 7687, **1998**.
52. NEWTON J.A., FRAY R.G. Integration of environmental and host-derived signals with *quorum sensing* during plant-microbe interactions. Cellular Microbiol. **6**, 213, **2004**.
53. VANNINI A., VOLPARI C., GARGOLGI C., MURAGLIA E., CORTESE R., DE FRANCESCO R., NEDDERMANN P., DI MARCO S. The crystal structure of the *quorum sensing* protein TraR bound to its autoinducer and target DNA. EMBO J. **21**, 4393, **2002**.
54. QIN Y.P., LUO Z.Q., SMYTH A.J., GAO P., VON BODMAN S.B., FARRAND S.K. *Quorum-sensing* signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. EMBO J. **19**, 5212, **2000**.
55. WATNICK P., KOLTER R. Biofilm, city of microbes. J. Bacteriol. **182**, 2675, **2000**.
56. COSTERTON J. W. Introduction to biofilm. Int. J. Antimicrobial. Agents. **11**, 217, **1999**.
57. HUBER B., RIEDEL K., HENTZER M., HEYDORN A., GOTSCHLICH A., GIVSKOV M., MOLIN S., EBERL L. The *cep quorum-sensing* system of *Brukholderia capacia* H111 controls biofilm formation and swarming motility. Microbiol. **147**, 2517, **2001**.
58. LABBATE M., ZHU H., THUNG L., BANDARA R., LARSEN M., WILLCOX M., GIVSKOV M., RICE S., KJELLEBERG S. *Quorum sensing* regulation of adhesion in *Serratia marcescens* MG1 is surface dependend. J. Bacteriol. **189**, 2702, **2007**.
59. LYNCH N.J., SWIFT S., KIRKE D.F., KEEVIL C., DODD C.E. WILLIAMS P. The regulation of biofilm development by *quorum-sensing* in *Aeromonas hydrophila*. Eviron. Microbiol. **4**, 18, **2002**.
60. STEIDLA A., ALLESEN-HOLM M., RIEDEL K., BERG G., GIVSKOV M., MOLIN S., EBERL L. Identification and characterization of an *N*-acylhomoserine lacton-dependent *quorum-sensing* system in *Pseudomonas putida* strain IsoF. Appl. Environ. Microbiol. **68**, 6371, **2002**.
61. CZACZYK K., MYSZKA K. Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation. Pol. J. Environ. Stud. **16**, 799, **2007**.
62. MYSZKA K., CZACZYK K. Characterization of adhesive exopolysaccharide (EPS) produced by *Pseudomonas aeruginosa* under starvation conditions. Curr. Microbiol. **58**, 541, **2009**.
63. TORRES P., MALAMUD F., RIGANO L., RUSSO D., MARANO M., CASTAGNANO A., ZORREQUIETA A., DOW J., VOJNOV A. Controlled synthesis of the DSF cell-cell signal is required of biofilm formation and virulence in *Xanthomonas campestris*. Environ. Microbiol. **9**, 2101, **2007**.
64. MARSHALL K.C. Biofilm: an overview of bacterial adhesion activity and control at surface. ASM News. **58**, 202, **1992**.
65. FRAY R.G. Altering plant-microbe interaction through artificially manipulating bacterial *quorum sensing*. Ann. Bot. **89**, 245, **2002**.
66. RASMUSSEN T.B., MANEFIELD M., ANDERSEN J.B., EBERL L., ANTHONI U., CHRISTOPHERSEN C., STEINBERG P., KJELLEBERG S., GIVSKOV M. How *Delisea pulchra* furanones affect *quorum sensing* and swarming motility in *Serratia liquefaciens* MG1. Microbiol. **146**, 3237, **2000**.
67. MANEFIELD M., WELCH M., GIVSKOV M., SALMOND G. P. C., KJELLEBRG S. Halogenated furanones from red alga, *Delisea pulchra*, inhibit carbapenem antibiotic synthesis and exoenzyme virulence factor production in the phytopathogen *Erwinia carotovora*. FEMS Microbiol. Lett. **205**, 131, **2001**.
68. TEPLITSKI M., ROBINSON J.B., BAUER W.D. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activity and affect population density-dependent behaviours in associated bacteria. Mol. Plant Microbe Interact. **13**, 637, **2000**.
69. JOINT I., TAIT K., CALLOW M.E., CALLOW J.A., MILTON D., WILLIAMS P., CÁMARA M. Cell-to-cell communication across the prokaryote-eukaryote boundary. Science. **298**, 1207, **2002**.
70. FRAY R.G., THROUP J.P., DAYKIN M., WALLACE A., WILLIAMS P., STEWART G.S.A.B., GRIERSON D. Plants genetically modified to produce N-acyl homoserine lactones communicate with bacteria. Nat. Biotechnol. **17**, 1017, **1999**.
71. MARKETON M.M., GONZALEZ J.E. Identification of two *quorum-sensing* systems in *Sinorhizobium meliloti*. J. Bacteriol. **184**, 3466, **2002**.